AGE DETERMINATION BY STAINING OTOLITHS OF *NOTOTHENIA ROSSII MARMORATA* WITH NINHYDRIN

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ABSTRACT. – A new technique of staining otoliths is described. Ninhydrin is able to dye in violet the successive circuli. This method gives the same ageing results than those obtained with the scales of *Notothenia rossii marmorata.*

RÉSUMÉ. – Une nouvelle technique de coloration des otolithes est décrite. L'utilisation de la ninhydrine permet d'obtenir la coloration en violet des annuli successifs séparés par des zones blanches. La méthode donne des résultats identiques à ceux obtenus par lecture des écailles chez *Notothenia rossii marmorata.*

Age reading by means of otoliths is a common method, but for many nototheniid fishes the application is difficult. Otoliths of *Notothenia rossii marmorata* are small and fragile. After brightening them with reagents (xylene, methyl benzoate, monobromnaphtalene and glycerine) a lot of secondary structures are to be seen which do not fit with the age determined from scales and bones (Freytag, 1979). Preparing thin slices from otoliths which had been embedded in polyester according to Rauck (1975), did not yield satisfactory results. The best success was obtained by treating the freshly taken otoliths with pure glycerine.

Staining techniques for otoliths are due to the different affinities of the dyeing reagents to the different chemical compositions of the annual rings. Albrechtsen (1968) e.g. used methyl violett B to stain the organic components. According to Dannevig (1956) there is a change in the composition of the concentric zones correlated with the different seasons. Especially the deposition of the chonchiolin varies seasonally.

On this basis we applied a protein staining technique for the age determination. Protein dyeing methods that require a very low pH are not applicable for dyeing complete otoliths. With ninhydrin dissolved in ethanol staining of material bearing NH_2 - groups is possible at neutral pH.

Otoliths which had been stored in glycerine were incubated in a 1 % solution of ninhydrin dissolved in 98 % ethanol. After 48 hours the slightly coloured otoliths were heated to 110°C for 15 minutes. After this treatment the layers rich in

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organic material assumed a deep violet colour and the otoliths were ready for reading. The ageing was carried out by stereo microscopy using transmittted polarized light. Some otoliths were broken through their center. These were watched in a drop of immersion oil under reflected light. The broken area showed sharply demarcated zones alternately white and violet from the periphery to the center which indicates that the staining solution had penetrated the whole otolith. For both ways of reading the otoliths were brought under immersion oil to compensate the rough and uneven surface especially that of the broken area. To get an even surface the breaking of the otoliths should be followed by polishing the cut surface.

It should be possible to store the otoliths in ninhydrin solution for an unlimited time after taking them from the fish without previously storing them in glycerine. But this has not been tried yet.

Up to now the number of rings in the otoliths of *N. rossii marmorata* corresponded well to the number of annuli in the scales and bones.

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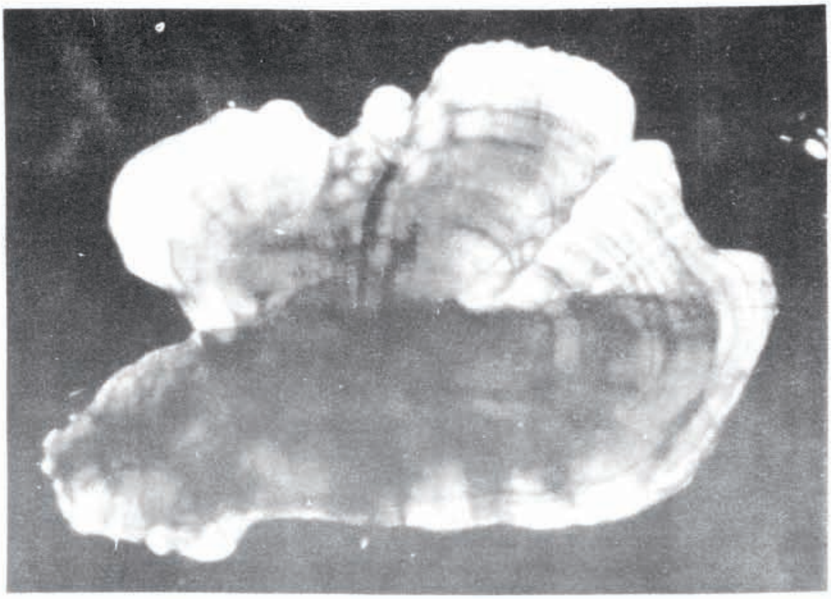

Fig. 1. – Otolith dyed with ninhydrin. *Notothenia rossii marmorata*, 39 cm, 6 years old.

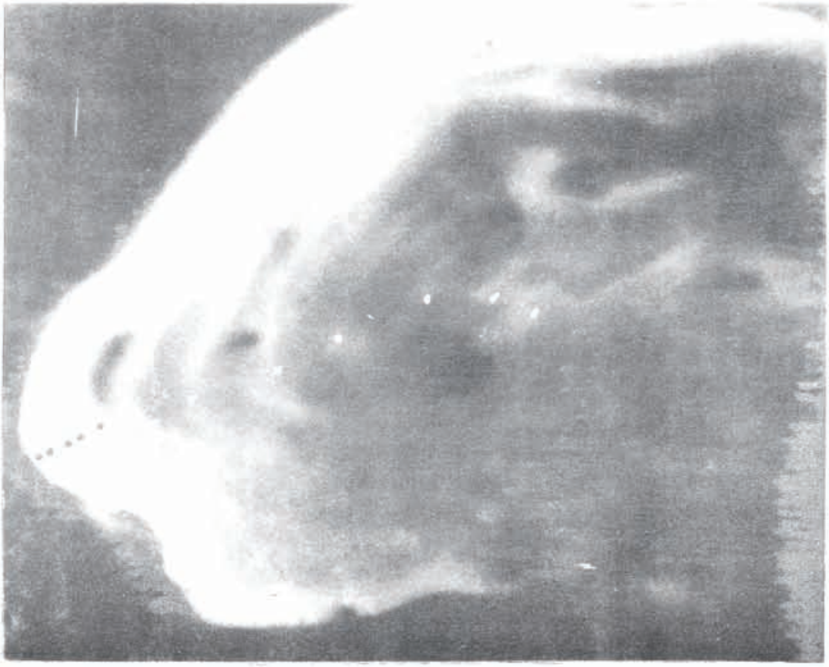

Fig. 2. – Broken area of an otolith dyed with ninhydrin. *Notothenia rossii marmorata*, 56 cm, about 12 years old.